THE OVIPOSITION BEHAVIOUR OF *AËDES AUSTRALIS* (ERICKSON) (DIPTERA, CULICIDAE).

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Synopsis.

The selection of an oviposition site by *A. australis* was influenced by the salinity of the water and by the physical properties of the surface of the water container, namely, its texture, reflectance and the presence of either a free, water surface or a moist, porous surface. Fresh water was preferred to a range of salinities, a rough surface to a smooth one, lower reflectances to higher reflectances, and a moist, porous surface to a free, water surface.

From a study of the interaction of these factors it was concluded that the preference for a moist, porous surface over a free, water surface so influenced the oviposition behaviour of this species that texture, reflectance and salinity had little effect upon it.

Introduction.

The occurrence of the larvae of *Aëdes australis* (Erickson) (= *concolor* Taylor) in the Sydney area in sandstone rock pools in which the salinity of the water varies (Mackerras, 1926; Woodhill, 1936) must be due to its oviposition behaviour being influenced by some factor of the environment other than salinity, because Woodhill (1941) has shown that fresh water is preferred to filtered sea water for oviposition.

Of the environmental factors which could influence the oviposition behaviour of *A. australis*, those investigated were the salinity of the water, the reflectance of the oviposition site, the texture of its surface, and whether the surface was moist and porous or a free water surface.

The factors reflectance, texture and either a moist, porous surface or a free, water surface have been found to influence the oviposition behaviour of *Aëdes scutellaris scutellaris* (Walker) (O'Gower, 1955), which breeds in tree holes and coconut husks (Penn, 1947); *Aëdes aegypti* (L.) (Beckel, 1955; O'Gower, 1957; Wallis, 1954*b*) which occurs in rain water storage tanks, etc. (O'Gower, 1956); *Aëdes scutellaris katherinensis* Woodhill (O'Gower, 1957) which presumably breeds in similar situations as *A. scutellaris scutellaris*; and *Aëdes albopictus* (Skuse) (O'Gower, 1957) which breeds in tree-holes and coconut husks (MacDonald, 1956). Salinity, however, usually has an inhibiting effect upon oviposition behaviour (Wallis, 1954*b*; Woodhill, 1941), but Mathis (1934) found *Aëdes meriae* (Ed. and Et. Sergent) (= *desbansi* (Seguy)) which occurs in salt water rock pools, preferred saline water to fresh water for oviposition.

Other environmental factors such as pH, organic pollution and vegetation have been found to influence oviposition behaviour (Lund, 1942; Manefield, 1951; Russell and Rao, 1942), but these were not studied.

Experimental Technique.

Pupae of *A. australis* were collected from salt water rock pools in the Sydney metropolitan area, and the emerged adults maintained in a controlled temperature and humidity room operating at 27 ± 1°C. and 75 ± 4 per cent. relative humidity, and with fluorescent light twelve hours (6 a.m. to 6 p.m.) in every twenty-four (Backhouse and O'Gower, 1956).

The mosquitoes were given selections between two oviposition sites, the surfaces of which varied in reflectance (black or grey), texture (rough or smooth), and either a moist, porous surface or a free, water surface. Either saline (one-half per cent. by weight sodium chloride) or tap water was used in these oviposition sites. A further range of salinities of one per cent., two per cent., and four per cent. saline was also used for some experiments. However, the combination of a saline solution and either a moist, porous surface or a free, water surface gave a choice between either a saline, moist, porous surface and a free, fresh, water surface; or between a fresh, moist, porous surface and a free, saline, water surface. In the former case there was an increase in salinity of the moist, porous surface due to evaporation, and in the latter case, because of an almost absolute preference for a moist, porous surface rather than a free, water surface (experiment 1), the influence of salinity could not be assessed. Therefore, as all combinations of the environmental factors involving both salinity and either a moist, porous surface or a free, water surface were either experimentally uncontrollable or else added little information on behaviour, they have been omitted from this paper except in experiment 17. However, because the texture of the surface has been found to be so important, and because the salinity of the water cannot be ignored in determining oviposition behaviour, the interaction of these two factors has been studied in further detail, by giving the mosquitoes selections between oviposition sites of different textures and salinities.

The oviposition sites in experiments 1, 2, 3, 8, 9, 15 and 17 were formed by placing into 9 cm. diameter Petri dishes either a pad of absorbent cellulose cotton with a filter paper of the required texture and reflectance on top of it, or a filter paper of the required reflectance on the bottom of the dish. Either tap water or saline was was then added to both containers, until the surface of the former was wet and glistening, and the water level in the latter was as high as the wet surface of the former. In experiments 4, 5, 6, 7, 10, 11, 12, 13, 14 and 16, the oviposition sites were formed by folding 11 cm. diameter filter papers into cones, placing them in 50 ml. beakers and adding either saline or fresh water until the water levels in the cones were half their vertical heights.

The filter papers* had surfaces which were (i) black and smooth, (ii) black and rough, (iii) grey and smooth, and (iv) grey and rough. Black "Tintex" dye was used to obtain papers of low reflectance. A "General Electric" spectrophotometer was used to measure the reflectances of these papers when wet. At a wave-length of 555 millimicrons the reflectances† of these papers when wet were: (i) black smooth, 3%; (ii) black rough, 3%; (iii) grey smooth, 7%; and (iv) grey rough 6%.

Seven replicates of each experiment were done, and the variances were calculated from the mean percentages of each. The significance of various preferences was estimated by means of the t-test.

Results.

1. *Comparison between free water and a moist surface.*—In experiment 1 the mosquitoes were given a choice between two water containers of similar reflectances. One dish had a free, water surface, the other a moist, porous surface. The moist, porous surface was significantly preferred ($p < 0{\cdot}001$) to the free, water surface (Table 1).

2. *The effect of reflectance.*—In experiment 2 the mosquitoes were given a choice between two water containers of similar textures but different reflectances. The preference for these containers was inversely related ($p < 0{\cdot}001$) to their reflectances (Table 1).

3. *The effect of texture.*—In experiment 3 the mosquitoes were given a choice between oviposition sites of similar reflectances but different textures. A rough surface was significantly preferred ($p < 0{\cdot}001$) to a smooth surface (Table 1).

4. *The effect of salinity.*—In each of experiments 4, 5, 6 and 7 the mosquitoes were given a choice between oviposition sites containing either fresh water or salinities of ½%, 1%, 2% or 4%. The fresh water was significantly preferred ($p < 0{\cdot}001$) to the full range of salinities (Table 1).

* (i) Whatman, No. 5; (iii) Allnutt and Sons, No. B1; (ii) and (iv) Allnutt and Sons, No. D3.

† Measured by C.S.I.R.O., National Standards Laboratory, University of Sydney.

TABLE 1.
Summary of Results.

Experiment Number.	Surface.	Re-flectance %	Number of Eggs Deposited.	Mean Percentage of Eggs Deposited.	Variance.	Value of "t".	Prob-ability.
A. The Influence of Water, Reflectance, Texture and Salinity on Oviposition by *A. australis*.							
1	Free water	7	95	2	3	100	<0·001
	Moist porous	7	4,598	98			
2	Black	3	4,422	68	2·5	50	<0·001
	Grey	7	2,146	32			
3	Rough	3	6,124	80	69	13·6	<0·001
	Smooth	3	1,621	20			
4	½% Saline water	7	4,291	33	24	13	<0·001
	Fresh water	7	7,443	67			
5	1% Saline water	7	2,558	20	3	65	<0·001
	Fresh water	7	10,080	80			
6	2% Saline water	7	877	12	5	63	<0·001
	Fresh water	7	6,686	88			
7	4% Saline water	7	504	4	3	99	<0·001
	Fresh water	7	10,256	96			

TABLE 2.
Summary of Results.

Experiment Number.	Surface.	Re-flectance %	Number of Eggs Deposited.	Mean Percentage of Eggs Deposited.	Variance.	Value of "t".	Prob-ability.
B. The Combined Influence of Water and Reflectance on Oviposition by *A. australis*.							
8	Black free water	3	53	2	1	183	<0·001
	Grey smooth moist porous	7	5,578	98			
C. The Combined Influence of Reflectance and Texture on Oviposition by *A. australis*.							
9	Grey rough	6	5,897	78	147	8·6	<0·001
	Black smooth	3	2,257	22			
D. The Combined Influence of Reflectance and Salinity on Oviposition by *A. australis*.							
10	Black ½% saline	3	8,100	66	31	10·6	<0·001
	Grey fresh water	7	3,906	34			
E. The Combined Influence of Texture and Salinity on Oviposition by *A. australis*.							
11	Rough ½% saline	3	12,562	73	38	13·3	<0·001
	Smooth fresh water ..	3	4,944	27			
12	Rough 1% saline	3	8,664	75	18	22·2	<0·001
	Smooth fresh water ..	3	2,985	25			
13	Rough 2% saline	3	8,737	78	8	37·2	<0·001
	Smooth fresh water ..	3	2,357	22			
14	Rough 4% saline	3	2,215	26	3	52	<0·001
	Smooth fresh water ..	3	6,290	74			

5. *The combined effects of reflectance and free water or a moist surface.*—In experiment 8 the mosquitoes were given a choice between two water containers, one with a free, water surface and a black background, the other with a grey, smooth, moist, porous surface. The grey, smooth, moist, porous surface was significantly preferred (p. $< 0{\cdot}001$) to the free, water surface (Table 2), but this preference was not different from that for a moist surface over a water surface of equal reflectance (experiment 1).

6. *The combined effects of reflectance and texture.*—In experiment 9 the choice was between a water container with a grey, rough, moist surface and a water container with a black, smooth, moist surface. The grey, rough surface was significantly preferred ($p < 0{\cdot}001$) to the black, smooth surface (Table 2), but this preference was not different from that for a rough surface over a smooth one, both of equal reflectance (experiment 3).

TABLE 3.

Summary of Results.

Experiment Number.	Surface.	Re-flectance %	Number of Eggs Deposited.	Mean Percentage of Eggs Deposited.	Variance.	Value of "t".	Prob-ability.
F. The Combined Influence of Texture, Reflectance and Water on Oviposition by *A. australis*.							
15	Black fresh water	3	87	2	2	127	<0·001
	Grey rough moist	6	7,181	98			
G. The Combined Influence of Texture, Reflectance and Salinity on Oviposition by *A. australis*.							
16	Black smooth fresh water ..	3	1,496	22	43	16	<0·001
	Grey rough saline water ..	6	7,155	78			
H. The Combined Influence of Texture, Reflectance, Water and Salinity on Oviposition by *A. australis*.							
17	Black fresh water	3	25	1	0	∞	<0·001
	Grey rough moist saline ..	6	7,404	99			

7. *The combined effects of reflectance and salinity.*—In experiment 10 the mosquitoes were given a choice between a water container with a black background with ½% saline in it and a container with a grey background with fresh water in it. The container with the black background holding ½% saline was significantly preferred ($p < 0{\cdot}001$) to the container holding fresh water and with a grey background (Table 2). However, this preference was not different from that for a black surface over a grey surface (experiment 2).

8. *The combined effects of salinity and texture.*—In each of experiments 11, 12, 13 and 14 the mosquitoes were given a choice between two water containers, one with a rough surface holding either ½%, 1%, 2% or 4% saline, the other with a smooth surface holding fresh water. In experiments 11, 12 and 13 the container with the rough texture holding either ½%, 1% or 2% saline was significantly preferred ($p < 0{\cdot}001$) to the smooth-surfaced container holding the fresh water. In experiment 14 the smooth-textured container holding fresh water was significantly preferred ($p < 0{\cdot}001$) to the rough-textured container holding 4% saline (Table 2).

9. *The combined effects of texture, reflectance and free water or a moist surface.*—In experiment 15 the mosquitoes were given a choice between two water containers, one with a free, water surface with a black background, the other with a grey, rough, moist, porous surface. The grey, rough, moist, porous surface was significantly preferred ($p < 0{\cdot}001$) to the free, water surface with the black background (Table 3).

However, this preference was not different from the preference for a moist surface over a water surface irrespective of texture or reflectance (experiments 1 and 8).

10. *The combined effects of texture, reflectance and salinity.*—In experiment 16 the mosquitoes were given a choice between a water container with a black, smooth surface holding fresh water and a container with a grey, rough surface holding ½% saline. The grey, rough-surfaced container holding saline water was significantly preferred ($p < 0.001$) to the black, smooth-surfaced container holding fresh water (Table 3). However, this preference was not different from the preferences for rough surfaces over smooth ones irrespective of reflectances (experiments 3 and 9).

11. *The combined effects of texture, reflectance, salinity and water or a moist surface.*—Because only 4% saline in a container with a rough-textured surface was less attractive than fresh water in a smooth-textured container (experiments 11, 12, 13 and 14), the mosquitoes were given a choice in experiment 17 between a fresh water surface with a black background and a 4% saline, moist, grey, rough surface. The saline, moist, grey, rough surface was significantly preferred ($p < 0.001$) to the fresh water surface with the black background (Table 3), but this preference was not different from that for a moist surface over a water surface (experiment 1), irrespective of reflectance (experiment 8), texture (experiment 15) or salinity (experiments 4, 5, 6 and 7).

Discussion.

Woodhill (1941) found *A. australis* preferred fresh water to a range of dilutions of filtered sea water, and the present investigation, using a range of dilutions of sodium chloride solutions, was in close agreement with this. Thus the preference for fresh water over saline water was due to a chemotactile response by the gravid females to sodium chloride in solution (see Wallis, 1954*b*, for a detailed investigation on the influence of salinity on oviposition).

However, because such behaviour could not explain the distribution of the larvae of this species in salt water rock pools, other factors of the environment were also studied. Thus, when the influences of the environmental factors of texture, reflectance, salinity and either a free, water surface or a moist, porous surface were compared, the preference for a moist surface over a water surface was found to be greater than the preferences for rough surfaces over smooth surfaces, for low reflectances over higher reflectances, and for fresh water over saline water.

Comparing the preferences of *A. australis* with those of *A. aegypti* (O'Gower, 1957) and *A. scutellaris scutellaris* (O'Gower, 1955) one finds:

	A. aegypti.	*A. australis.*	*A. scutellaris.*
Preference for rough over smooth	59% to 41%	80% to 20%	60% to 40%
Preference for black over grey	73% to 27%	68% to 32%	70% to 30%
Preference for water over moist	75% to 25%	2% to 98%	54% to 46%
Preference for fresh over saline	74% to 26%*	67% to 33%	Not available

* Mean of percentages from O'Gower (unpublished), Wallis (1954*b*) and Woodhill (1941).

It can be seen from these figures that while the preference for a water surface over a moist surface was only slight for *A. scutellaris*, with *A. aegypti* the preference was most decided, but with *A. australis* the preference was reversed and almost absolute. This difference in behaviour of the two former species appears to be correlated with their different larval distributions (O'Gower, 1957). However, the similarity in behaviour of *A. aegypti* and *A. australis* with regard to salinity cannot be correlated with their very different larval habitats.

A. meriae breeds in salt water rock pools and, because it lays its eggs on the surface of the water (Mathis, 1929), the preference for salt water over fresh water (Mathis, 1934) is understandable. *A. australis* breeds in the Sydney area only in sandstone, salt water, rock pools (Mackerras, 1926) and its eggs have been found on the rock surface by the author either at or above the water line. Thus the almost absolute preference for a moist surface over a water surface, and the decided preference for a rough surface over a smooth one, help explain the unimportance of salinity in the oviposition behaviour of *A. australis.*

This explanation becomes more logical when the interaction of these environmental factors is studied, for the attractiveness of a rough surface masks both the unattractiveness of saline water (except when the salinity equals that of sea water) and the attractiveness of lower reflectances (experiments 11, 12, 13, 14 and 9), whilst the preference for a moist surface over a water surface so influences oviposition behaviour that texture, reflectance and salinity do not affect it (experiment 17).

Such behaviour can explain the occurrence of the larvae of *A. australis* in salt water rock pools, but not their absence from fresh water rock pools, and although it is possible that other environmental stimuli may influence the selection of an oviposition site by this species, this investigation was limited to a study of the influence of salinity and of the physical properties of the surface of a water container upon the oviposition behaviour of *A. australis.*

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